

Date: August 30, 2001 Label No. EL856453485US

I hereby certify that, on the date indicated above, I deposited this paper or fee with the U.S. Postal Service and that it was addressed for delivery to the Assistant Commissioner for Patents, Washington, DC 20231 by "Express Mail Post Office to Addressee" service.

Sandra Stocklinski

Name (Print)

Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Koji KISHI et al.

Application No.: Unassigned

Group Art Unit: Unassigned

Filed: August 30, 2001

Examiner: Unassigned

For: METHOD FOR ASSAYING BIOLOGICAL SAMPLE COMPONENT

PRELIMINARY AMENDMENT

The Assistant Commissioner for Patents  
Washington, D.C. 20231

August 30, 2001

Sir:

Prior to calculation of the filing fees and examination of the above-identified application on the merits, applicants respectfully request that the application be amended as follows:

**IN THE CLAIMS:**

Please cancel claims 8, 9, 10, 11, 12, and 13 without prejudice or disclaimer of the subject matter therein.

Please substitute the following amended claims for the pending claims with the same numbers in the above-identified application. (A version of the amended claims with markings to show the changes made is also attached.)

1. (Amended) A method for assaying a specific component in a lipoprotein fraction in a serum by an enzymatic reaction, which comprises introducing a controlling means which is established by selecting the enzymatic reaction, for enabling an enzymatic reaction preferentially with respect to an object component in the specific lipoprotein fraction without forming complexes nor aggregates, thereby specifically assaying the component.

Please add the following new claims:

--14. The method for assaying a specific component in a lipoprotein fraction according to claim 5, wherein said controlling means is a means for controlling ionic strength of a reaction liquor so as to facilitate the enzymatic reaction of the target component in the specific lipoprotein fraction in the reaction liquor; and a) said controlling ionic strength increases the ionic strength of the reaction liquor to a sufficiently high level so as to facilitate the enzymatic reaction of the component in a high-density lipoprotein (HDL) in the liquor; or b) a nonionic surfactant that has reaction selectivity to the HDL fraction and an HLB value of 16 or more is used as said nonionic surfactant, thereby enabling the enzymatic reaction directly and/or preferentially with respect to the component in the HDL fraction in the reaction solution, or both a) and b) are carried out in combination.

15. The method for assaying a specific component in a lipoprotein fraction according to claim 4, wherein a) said controlling means is a means for controlling ionic strength of a reaction liquor so as to facilitate the enzymatic reaction of the target component in the specific lipoprotein fraction in the reaction liquor or b) said controlling means is a means for enabling the enzymatic reaction directly and/or preferentially with respect to the component in the specific lipoprotein fraction in the reaction liquor, utilizing reaction selectivity of a selected nonionic surfactant to the specific lipoprotein, or both a) and b) are carried out in combination.

16. The method for assaying a specific component in a lipoprotein fraction according to claim 14, wherein said method is a method for assaying cholesterol in an LDL fraction, which comprises introducing a means for selectively subjecting a cholesterol component in an HDL fraction to an enzymatic reaction to assay or digest thereof in the first enzymatic reaction system, and said method further comprising subjecting the cholesterol component in the LDL fraction to an enzymatic reaction in a second enzymatic reaction system by utilizing said assay method again, wherein said controlling means is a means for enabling the enzymatic reaction directly and/or preferentially with respect to the component in the specific lipoprotein fraction in the reaction liquor, utilizing reaction specificity of an enzyme to the specific lipoprotein and using a nonionic surfactant that has an HLB value of 11 to 13.

17. The method for assaying a specific component in a lipoprotein fraction according to claim 15, wherein said method is a method for assaying cholesterol in an LDL fraction, which comprises introducing a means for selectively subjecting a cholesterol component in an HDL fraction to an enzymatic reaction to assay or digest thereof in the first enzymatic reaction system, and said method further comprising subjecting the cholesterol component in the LDL fraction to an enzymatic reaction in a second enzymatic reaction system by utilizing said assay method again, wherein said controlling means is a means for enabling the enzymatic reaction directly and/or preferentially with respect to the component in the specific lipoprotein fraction in the reaction liquor, utilizing reaction specificity of an enzyme to the specific lipoprotein and using a nonionic surfactant that has an HLB value of 11 to 13.

18. The method for assaying a specific component in a lipoprotein fraction according to claim 16, wherein said method is a method for assaying cholesterol in a VLDL (very low-density lipoprotein) fraction, which comprises simultaneously or separately treating said first enzymatic reaction system and said second enzymatic reaction system in said assay method to have the cholesterol component remained and then introducing a means for decomposing the VLDL fraction to subject the cholesterol component in the VLDL fraction to an enzymatic reaction.

19. The method for assaying a specific component in a lipoprotein fraction according to claim 17, wherein said method is a method for assaying cholesterol in a VLDL (very low-density lipoprotein) fraction, which comprises simultaneously or separately treating said first enzymatic reaction system and said second enzymatic reaction system in said assay method to have the cholesterol component remained and then introducing a means for decomposing the VLDL fraction to subject the cholesterol component in the VLDL fraction to an enzymatic reaction.

20. The method for assaying a specific component in a lipoprotein fraction according to claim 14, further comprising a step for adding cholesterol oxidase or cholesterol dehydrogenase to digest free cholesterol.